

RESEARCH ARTICLE

URINE CYTOLOGY AMONG HIV-SEROPOSITIVE PERSONS

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ABSTRACT: Human Immunodeficiency Virus (HIV) infection and the use of antiretroviral therapy (ART) are risk factors in the development of urinary system neoplasia. In this study, the urine smears of HIV-seropositive persons were screened for atypical epithelial changes. A total of 61 subjects, comprising 41 HIV-seropositive persons (20 ART users and 21 non-ART users) visiting the University of Calabar Teaching Hospital and Immanuel Infirmary in Calabar and 20 HIV-seronegative persons from the general public between the ages of 18 to 45 years old of both sexes participated in the study. Fresh voided urine samples were assessed with Meditest Combi-9 urinalysis strip and cytologically using Papanicolaou method. Results showed that age group 32-38 years and 18-24 years had the highest number of participants among the non-ART users 12(57.1%) and ART users and control subjects 13(65.0%) respectively. There was statistical significance between the non-ART users ($p=0.001$) and ART users ($p=0.001$) with control. Females were associated more than males among the non-ART users 16(76.2%), ART users 16(80%) ($p=0.002$). The urinalysis result showed that only the ART users had 2(10%) abnormal presence of blood. The cytological findings of abnormal epithelial cells were associated with the non-ART 1(4.8%), ($p=0.001$) and ART 2(10%), ($p=0.001$) users respectively. The urine smears showed benign atypical, keratinized and degenerated superficial epithelial cells. Therefore, urine cytology examination has revealed the presence of atypical epithelial cell changes among HIV-seropositive persons. It can be a useful tool in the management of HIV treatment and progression and is recommended for their routine follow-up.

KEYWORDS: HIV, urine cytology, ART, Calabar, Papanicolaou stain, urinalysis

INTRODUCTION:

The increasing spread of HIV worldwide led to increase in morbidity and mortality due to low immunity from the diseases until the introduction of antiretroviral therapy. In Nigeria, a prevalence of 3.2 per cent between 15-49 years occurred with an estimated death of 210,000 in 2013.¹ HIV

decreases the immune system causing diseases in almost all organs of the body including the urinary system.^{2,3} kidney diseases are the most common and the major risk factors are high viral load, low CD4 count, fluid and electrolyte imbalances and the use of antiretroviral drugs.⁴ Bladder cancer

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has also been associated with the disease.^{5,6} With the advent of ART, the quality of life of people living with HIV was improved by suppressing viral load, reducing opportunistic infections, and restoring the immune system.^{7,8} However, their administration has been met by several toxicities to the urinary system such as Falconi syndrome, glomerular sclerosis, acute tubular necrosis, nephropathy, urinary tract problems, end stage renal disease,^(5,9) and neoplasia.^{5,6}

Due to the possible side effects of ART and cytopathic effect of HIV, current follow-up screening parameters for HIV/AIDS patient's management have been the assessment of renal function,¹⁰ based on urinalysis and assay of electrolytes, urea, and creatinine. As at present, there is no policy to carry out urine cytological screening for HIV patients. Thus, this study was carried out to evaluate the urine cytology of HIV patients for atypical cells and associate these findings with either the HIV infection or the use of ART or both. This will serve as a baseline study for further research and better patient management.

MATERIALS AND METHODS:

Study area

The study was carried out in two health facilities, the University of Calabar Teaching Hospital (UCTH) and Immanuel Infirmary in Calabar, Cross River state. The two hospitals are located within Calabar metropolis and are sites for treatment and care for persons living with HIV.

Study subjects and data collection:

A total of 61 subjects between the ages of 18 to 45 years old of both sexes gave consent to participate in the study. The test subjects were 41 HIV-seropositive persons (comprising 20 ART users and 21 non-ART users) visiting the University of Calabar Teaching Hospital (UCTH) and Immanuel Infirmary in Calabar. The control subjects were 20 HIV-seronegative persons drawn from the general public. A questionnaire was used to obtain demographic data, history of HIV infection, duration of ART use and history of UTI from the subjects.

Inclusion criteria: The test subjects were HIV-seropositive persons who were non-ART users and those that were ART users. While the control group were subjects that were HIV- seronegative.

Exclusion criteria: Pregnant women and people with a history of diabetes and UTI were excluded from the study.

Sample collection:

Fresh voided 61 urine samples from each subject were collected into sterile universal containers. The macroscopic appearances of the urine samples were assessed before being subjected to urinalysis and cytological analysis using Papanicolaou staining technique.

Urinalysis:

The Medi-Test Combi-9 urine dipstick strip (Macherey-Nagel Duren, Germany) was used to carry out urinalysis based on manufacturer's instruction. The urinalysis strip was dipped into the urine sample after gently mixing. The result was read immediately, following comparison with the colour chart on the urinalysis strip container.

Papanicolaou Staining Technique:

Urine smears were fixed in 95% ethanol for 15 minutes. The smears were stained with Papanicolaou stain according to method of Bancroft and Gamble¹¹. The stained slides were examined microscopically with a light microscope using x40 objective.

Statistical analysis:

Data collected and results were analyzed using Statistical Package for Social Sciences (SPSS) version 20 (Armonk, New York: IBM Corporation). The results were expressed as percentages. Prevalence was calculated using a 2x2 contingency table. Student t-test of independent samples was used to test the association with gender. Results were statistically significant at probability level of less than or equal to 0.05 ($P \leq 0.05$).

RESULTS:

The age distribution of the subjects is shown in Table 1. Age group 32-38 years had the highest number of participants 12(57.1%) among the non-ART users while age group 18-24 years had the highest number of participants 13(65.0%) among the ART users and the control subjects. The age distribution was statistical significance between the non-ART users and control ($\chi^2=63.000$, $p=0.001$) and ART users and control ($\chi^2=40.00$, $p=0.001$).

Figure 1 shows the distribution of the subjects by gender. Females accounted for the highest number of participants among the non-ART users 16(76.2%), ART users 16(80%), and control 16(80%) than males who had non-ART users 5(23.8%), ART users 4(20%), and control 4(20%). The gender distribution was not statistical significance between the non-ART users and control ($\chi^2=0.087$, $p=0.768$) and ART users and control ($\chi^2=0.000$, $p=1.000$). There was statistical significance between the males and females among the non-ART users and ART users ($t=23.000$, $p=0.002$).

Figure 2 shows the normal urinalysis outcome of subjects. The non-ART users and control had 100% absence of abnormal constituents in all the 9 reagent areas of the Combi-9 strip. The ART users had 2(10%) abnormal presence of blood and 18(90%) absence of abnormal constituents in the urine.

The cytological findings of subjects are shown in Table 2. Among the non-ART users 1(4.8%) had abnormal epithelial cells, the ART users had 2(10%) cases of abnormal epithelial cells and no abnormality was found among the control subjects 0(0%). There was statistical significance between the non-ART users and control ($\chi^2=17.190$, $p=1.000$) and ART users and control ($\chi^2=12.800$, $p=1.000$) based on cytological findings.

Figure 3 shows the photomicrographs of urine cytology of Non-ART and ART users. The Papanicolaou-stained urine smear of the non-ART user shows benign atypical epithelial cells characterized by mild perinuclear halo (H) and benign nuclei (N). Smear of the ART user shows normal superficial epithelial cell (N), superficial

cell with keratinization (K) and eosinophilic cytoplasm and another undergoing degenerative change with marked loss of cytoplasm (D).

Table 1: Age distribution of subjects

Age (years)	Non-ART users No. (%)	ART users No. (%)	Control No. (%)
18-24	1 (4.8)	13 (65)	13 (65)
25-31	5 (23.8)	6 (30)	6 (30)
32-38	12 (57.1)	1 (5)	0 (0)
39-45	3 (14.3)	0 (0)	1 (5)
Total	21 (100)	20 (100)	20 (100)
	$\chi^2=63.000$, $p=0.001$	$\chi^2=40.000$, $p=0.001$	

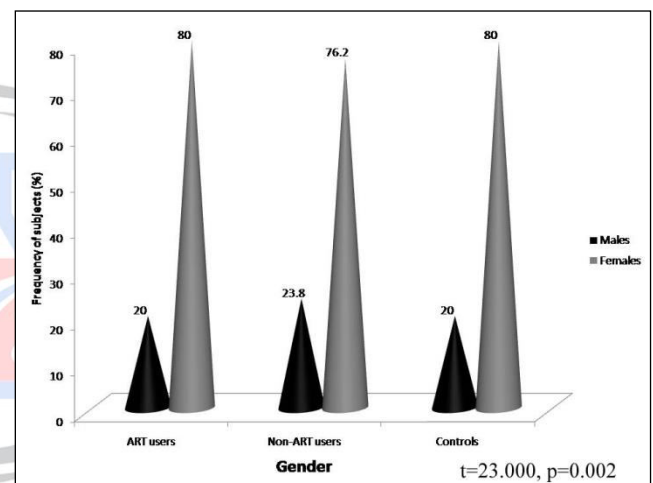


Figure 1: Distribution of subjects by gender

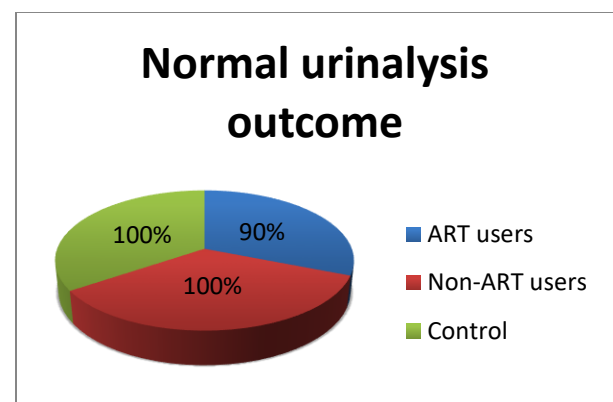
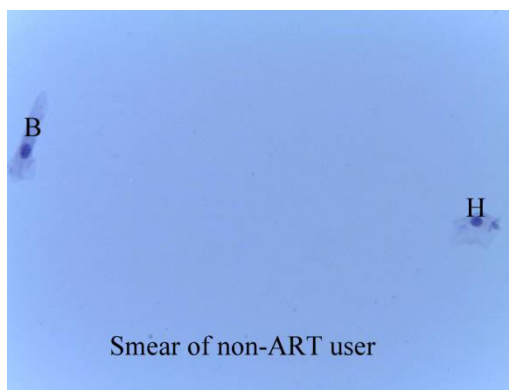


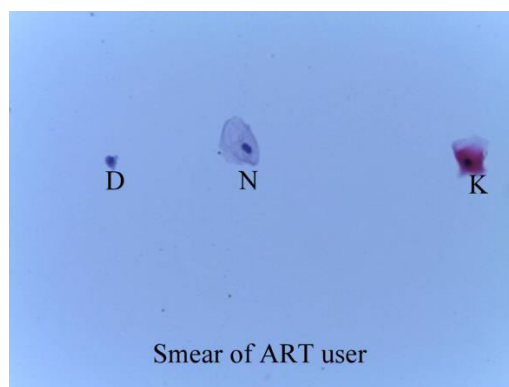
Figure 2: Normal urinalysis outcome of subjects

Table 2: Cytological findings of subjects

Cytology	Non-ART users	ART users	No. of Control
Normal No.(%)	20 (95.2)	18 (90)	20 (100)
Abnormal No.(%)	1 (4.8)	2 (10)	0 (0)
Total No.(%)	21 (100)	20 (100)	20 (100)
	($\chi^2=17.190$, p=0.001)	($\chi^2=12.800$, p=0.001)	



3A:Non-ART-users



3B: ART users

Figure 3: Photomicrographs of urine cytology of Non-ART and ART users. Plate 3A shows two benign atypical superficial squamous cells characterized by mild perinuclear halo (H) and

benign nuclei (N). Plate 3B is smear of ART user showing normal superficial epithelial cell (N), superficial cell with keratinization (K) and eosinophilic cytoplasm and another undergoing degenerative change with marked loss of cytoplasm (D). (Papanicolaou x400)

DISCUSSION:

Papanicolaou-stained urine cytology has been a useful method in screening of urinary tract diseases especially in detecting atypical epithelial changes. In this study, the urine smears of HIV-seropositive persons (both non-ART and ART users) and HIV-seronegative controls were screened for atypical epithelial changes. This is due to the fact that HIV infection has been associated with several diseases of the urinary system.^{10,12}

The majority of the test subjects on ART were mostly young adults 18-24years old. This is similar to report by NACA¹³. The reason behind this observation is attributed to the likely high sexual behaviors of young persons NACA¹³. Also females accounted for the highest number of infected persons than the males. This is due to the fact that females are the most at risk persons because of early age of first sexual intercourse, multiple sexual partners, and poor economic background as stated by NACA¹³.

The urinalysis results showed the absence of abnormal chemical constituents in the urine except for 2(10%) blood found among the ART users. Similar report of haematuria was reported by Mukherjee et al.⁵ and Gaughan et al.⁶ had stated that haematuria is a risk factor for bladder cancer development. This shows that these ART users may be at risk of bladder cancer. There was absence of proteinuria among the subjects which points to increase risk of nephropathy and this correlates with Mukherjee et al.⁵ and Offor and Enyinna,¹⁴.

Finally, the cytological findings of the non-ART user showed benign atypical epithelial cells with mild perinuclear halo and benign nuclei. While the smear of the ART user showed superficial cell with keratinization and another undergoing degenerative change with marked loss of cytoplasm. This finding reveals that the epithelial

cells exposed to HIV infection and ART may gradually undergo atypical cellular changes that may lead to neoplasia. Siddappa et al.¹⁵ and Mukherjee et al.⁵ had reported similar findings of cellular atypia and abnormal cytology.

The mechanisms behind the development of cellular atypia and neoplasia are as a result of the interaction of the HIV viral antigen with the host cell. The use of ART on the other hand leads to nephrotoxicity such as mitochondria toxicity, abnormalities of transport proteins of the epithelial cells, and tubular necrosis.¹⁰

CONCLUSION:

Urine cytology examination has revealed the presence of atypical epithelial cell changes among HIV-seropositive persons. It can be a useful tool in the follow-up and management of HIV treatment and progression along side routine renal function tests like serum analysis for electrolytes, urea, creatinine, and urinalysis. Therefore, urine cytology is recommended for the routine screening of persons living with HIV whether placed on ART regimen or not.

Ethical approval:

Ethical approval was sought and gotten from the Cross River State Ministry of Health Research Ethics Committee with approval number Rec./2015/340. Informed consent was sought from the participants before commencement of the study.

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